REMARKS

Applicants respectfully request entry of substituted pages 12, 48 and 49 as amended and corrected Figure 14. The errors were typographical in nature and were not detected prior to the filing of the Application. Support for these amendments can be found throughout the specification and the claimed subject matter. For example, in the preferred embodiments, Applicants disclose "0.01% to about 30% (w/w) of Coenzyme Q10" (page 2, lines 3-5; page 2, lines 10-12; page 2, lines 19-21; page 3, lines 1-2; page 3, lines 14-16; page 3, lines 29-30; page 13, lines 13-15). Effective amounts are disclosed on page 32, lines 15-18. Further support for the amendments is shown in Figures 1-10 and 16-27. The amendments do not go beyond the disclosed subject matter and are obvious in the face of the support in the specification. No new matter has been added by virtue of these amendments and their entry is respectfully requested.

CONCLUSION

Applicants respectfully request entry of substituted pages 12, 48 and 49 of the specification and substitute Figure 14. Applicant invites the Examiner to call the undersigned if it is believed that a telephone interview would expedite entry of the afore-mentioned amendments.

The US/RO is hereby authorized to charge the amount due for any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing or during prosecution of this application to Deposit Account No. 50-0951.

Respectfully submitted,

Date: December 13, 2005

Nicholas A. Zachariades
Reg. No. 56,712
AKERMAN SENTERFITT
Customer No. 30448
P.O. Box 3188
West Palm Beach, FL 33402-3188
Telephone: (561) 653-5000

Docket No. 7230-21WO

By the term "modulate," it is meant that any of the mentioned activities, are, e.g., increased, enhanced, increased, agonized (acts as an agonist), promoted, decreased, reduced, suppressed blocked, or antagonized (acts as an agonist). Modulation can increase activity more than 1-fold, 2-fold, 3-fold, 5-fold, 10-fold, 100-fold, etc., over baseline values. Modulation can also decrease its activity below baseline values.

As used herein, the term "selective for tumor cells" refers to the effects of the Coenzyme Q10 pharmaceutical compositions, such as inhibition of tumor growth, apoptosis, anti-angiogenic effects and which are not detectable when applied to normal cells, as described in detail in the examples which follow.

CoQ10 Compositions

In a preferred embodiment, the invention provides CoQ10 compositions for the treatment of cancer. Preferably, the compositions comprise at least about 1% to about 25% CoQ10 w/w, more preferably, between about 1% to about 20% CoQ10 w/w. In the representative embodiment described in the Examples section below, a topical formulation of CoQ10 is applied to the skin of a tumor-bearing animal to reduce the growth rate of the tumor. CoQ10 can be obtained from Pure Prescriptions (San Diego, CA) in powdered form in any suitable quantity (e.g., 1 kilogram). To deliver a CoQ10-containing composition, any suitable carrier can be used. Liposomes, for example, may be used as a carrier. An exemplary liposomal formulation is composed of Phospholipon 90G (American Lechitin, Stanford, CT), Phospholipon 90H (American Lechitin, Stanford, CT), Glycerol, Butylated hydroxytoluene (BHT), Ethanol, Medium Chain Triglycerides (MCT), lavender (Sigma-Aldrich, St. Louis, MO) and Coenzyme Q10 (Pure Prescriptions, San Diego, CA). An example of a protocol for preparing this formulation entails first dissolving 10 g of Phospholipon 90H, 5g Phospholipon 90G, with 1.5 g MCT, 0.3g BHT, and 9 ml of ethanol at 75 °C. Next, 1.1 g of Coenzyme Q10 are dissolved into the mixture. 65 ml of 1 mM phosphate buffer (pH 8.2) prepared with nitrogen saturated water, 13.3 g glycerol, and 50 µL of lavender are added. The above mixture is blended in a high-speed blender at 12,000 RPM to form a cream. The cream is stored at 4 °C until used.

Subjects

Because subjects from many different species have tumors and are susceptible to acquiring a tumor, the invention is compatible with many types of animal subjects. A non-exhaustive exemplary list of such animals includes mammals such as mice, rats, rabbits,

- 15 mL Centrifuge Tubes

- Coulter Counter Vials (Beckman Coulter Inc.)
- 0.05% trypsin (Cat# 25-052-C1- 1X Trypsin-EDTA, Cellgro)
- Centrifuge tubes (2mL)
- Anesthetic (Aventin)

Procedures:

Subculture flasks as per the cell subculturing protocol described above. After aspirating supernatant, combine pellets from each flask diluted slightly with PBS with a 5mL pipette. Dilute final cell suspension to contain approximately ten million cells per 100µL. Transfer cell suspension to micro-centrifuge tubes (2mL). Place in ice immediately and leave in ice until injected. Anesthetize mice via an intraperitoneal injection with 0.3cc Aventin. Inoculate each animal subcutaneously with 0.1cc cell suspension per site. Transfer any remaining cells into a 15mL centrifuge tube. Dilute to 10mL with medium. Centrifuge at 2500 RPM for 8 minutes. Aspirate supernatant. Add 10mL media to centrifuge tube. Create a homogenous cell suspension by pipetting and vortexing. Seed cells in a T75 flask to ensure experimental cell viability.

Example 2 – Effect of A Topical Formulation Of Coenzyme Q10 on SK-MEL28 Tumors In Mice

Melanoma tumors were induced in mice by SK-MEL28 injection into the subcutaneous layer. The animal study consisted of both a control and treatment group each containing four mice. The mice were inoculated with two tumors and the graph of Figure 14 represents the resulting mean mass for the tumors in each mouse. A topical formulation of Coenzyme Q10 (A 1.0% and 1.5% formulation was tested) was applied to the tumors in the treatment group daily for a period of 30 days. After which, the tumors were excised and the mass was determined. The difference in the overall mean mass of the treatment group was significant compared to the control ($P<0.05$).

Example 3-Preparation of Topical CoQ10 Cream

Reagents:

-Phospholipon 90G (American Lechitin, Stanford, CT)

-Glycerol

-BHT

-Ethanol

-MCT

-lavender (Sigma-Aldrich)

-CoQ10 (Pure Prescriptions, San Diego, CA)

Procedure:

10g of Phospholipon 90G and 5g of Phospholipon 90H (American Lechitin, Stanford, CT) was dissolved in a mixture of 13.3g of Glycerol (Sigma-Aldrich, St. Louis, MO), 0.3g BHT (Sigma-Aldrich), 9ml ethanol (Sigma-Aldrich), and 1.5g MCT (Sigma-Aldrich) at 60°C. 1.1g of CoQ10 (Pure Prescriptions) were dissolved into the resulting mixture. 65ml of 1mM phosphate buffer (pH 8.2) prepared with nitrogen saturated water and 0.2ml of lavender (Sigma-Aldrich) were added and the mixture was blended in a high speed blender at 12,000 RPM to form a cream. The cream was stored at 4°C until used.

Example 4: Apoptosis Analysis for JC-1 Stain

Apoptosis was measured using a mitochondrial membrane dye JC-1, 5,5',6,6'-tetrachloro-1,1' ,3,3' -tetraethyl- benzimidazolylcarbocyanine chloride (Molecular Probe, Eugene, OR). Treatments consisting of DMEM-F12 media supplemented with 1X PSA, 5% FBS and 0, 50, 100, and 200 μM concentrations of coenzyme Q10 were prepared in 60 x 15 mm tissue culture dishes (Costar- Cambride, MA). PC-3 cells were seeded at 500,000 cells per dish and incubated for 24 hours. The cells were trypsinized using 2mL trypsin-EDTA and subjected to centrifugation at 2,500 rpm for 8 minutes. They were resuspended in 1 mL of Ham's F12 medium lacking serum and phenol red (Cascade Biologics, Inc-Portland, OR) and promptly placed on ice. A 1 mg/ml stock solution of JC-1 was prepared using sterile DMSO and 10 μL was added to each cell suspension while gently vortexing. The cells were incubated at 37°C for 15 min, diluted with 4 ml of Ham's F12 medium and centrifuged at 600 rpm for 7 min. Resuspended in 5 ml of cold PBS (Gibco-Grand Island, NY), the cells were centrifuged again at 600 rpm for 7 min. The cell pellet was then suspended in 1 ml of cold PBS and transferred to nylon filter top flow cytometry tubes covered with foil to prevent light penetration. The samples were analyzed by flow cytometry for changes in uptake of fluorescent dye. The monomer JC-1 displays green fluorescence (λ_{em}= 527 nm) while the J-aggregates display red fluorescence (λ_{em} = 590 nm). Permeabilized mitochondria accumulate the JC-1 monomer dye prior to and during apoptosis.

Other Embodiments